Wilson, J. F. and W. K. Bates. Ethylene glycol treatment of Neurospora conidio.

Recently we described some effects of treatment of Neurospora conidio with theylene glycol (Bates and Wilson 1972 Genetics 68: s4). This treatment results in conidio which enlarge, with concomitant weight gain, and which become osmotically sensitive after two or more days. Osmotic disruption of these cells yields large numbers of intact nuclei and mitochondria, while gradual removal of the ethylene glycol results in approximately 75% germination within one hour. We now present some details of the methodology involved.

The conidio routinely used ore from seven-to-fwrtecn-day-old cultures, grown at 30°C on Vogel's minimal agar medium, with supplements as required for mutants. The strain used for most of our studies is a m-isolate of the Oak Ridge wild type. Additional studies with me-3 (36104) FGSC#502, inos (37401) FGSC#406, and [mi-1] (poky, mi-I-1.8) FGSC#1578, with appropriate supplements, have indicated that the effect is not limited to one strain, although variations do occur in the degree of the response.

Conidia ore harvested in sterile water, filtered through four layers of sterile gauze to remove hyphol fragments, and the concentration is determined with a hemacytometer. The conidial suspension is allowed to stand for at least one hour a+ 25°C before the conidia ore transferred to ethylene glycol medium. This pretreatment with water results in foster and more uniform enlargement of the conidia in response to ethylene glycol. Pre-treatment periods longer than one hour produce no additional effect,

The formulation for 100 ml of the ethylene glycol medium is: 2 ml of 50X Vogel's minimal medium; 80 ml of distilled water; 18 ml ethylene glycol, reagent grade (20 grams); 1.5 g sucrose. We routinely double these amounts to obtain 200 ml ethylene glycol medium, and use this volume in 500 ml Erlenmeyer type flasks with stainless steel closures (DeLong culture flasks). All components are autoclaved together in the flask.

We inoculate at 1-3x Id conidio per ml medium (2-6x 10^9 per flask) by centrifuging the volume of aqueous suspension of conidia necessary for each flask in a sterile screw-copped tube and decanting the water from the conidiol pellet. The conidio ore then re-suspended in a part of the contents of a flask of ethylene glycol medium and transferred bock to the flask. Thus, inoculation is achieved without dilution of the medium. Flasks ore then placed on a rotary shaker at 25°C with carriers mounted at a 15° angle and ore shaken continuously at 150 rpm. Osmotic sensitivity is demonstrable at 48 hrs, and both size and osmotic sensitivity continue to increase for at least IO days. We hove observed more than 80% viability after 8 days of this treatment.

Osmotic disruption is accomplished by centrifuging a suitable portion of the suspension ond re-suspending the pellet in a hypotonic solution to approximately 10% of the original volume. Disruption occurs within a few seconds. For mitochondrio, the pellet is resuspended in 2% sucrose-1mM EDTA at 5% of the original volume, followed by on equal volume of 28% sucrose-1mM EDTA at 30 seconds. It should be noted, however, that such mitochondrio ore not identical to those prepared by sand grinding.

For studies involving germination (including sorbose plating) it is necessary to dilute the ethylene glycol gradually, allowing the conidio to equilibrate at the lower concentrations. We hove accomplished this with minimal disruption by non-linear rates of addition of water or minimal Tedium, according to the following schedule:

10 ml conidiol suspension in 20% ethylene glycol in 125 ml Erlenmeyer flask on magnetic stirrer at room temperature.
Add diluent at 1 ml/min for IO min to yield 10% solution,
Add diluent at 2 ml/min for IO min to yield 5% solution,
Add diluent-at 4 ml/min for 15 min to yield 2% solution.

Diluent is added by.a peristaltic pump, aseptically if necessary. If faster dilution is required, rates of addition con be doubled with only a slight increase in disruption.

Ethylene glycol treated conidio ore much more susceptible to disruption by sand grinding than ore untreated conidia, as judged by comparative extraction yields. This allows preparation of extracts when osmotic shock is not desirable, or in the preparation of mitochondrio. The procedure is: dilute as described above; centrifuge to concentrate the conidio; re-suspend in the extraction medium; and grind with sand with a mortar and pestle.

Although various modifications will be necessary to suit specific experiment.1 conditions, the methods outlined above should prove adequate for preliminary studies. A more complete characterization of these conidio and the extracts obtained from them will be presented elsewhere. - - - (Supported by grants from the Research Corporation and the UNC-G Research Council) - - -
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